(*NEW SERIES.*)

No. 8.

SCIENTIFIC MEMOIRS

BY

OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

GOVERNMENT OF INDIA.

A PRELIMINARY REPORT ON A PARASITE FOUND IN PERSONS SUFFERING FROM ENLARGEMENT OF THE SPLEEN IN INDIA.

BY

LIEUT. S. R. CHRISTOPHERS, M.B., I.M.S.

(*On special duty.*)

ISSUED UNDER THE AUTHORITY OF THE GOVERNMENT OF INDIA
BY THE SANITARY COMMISSIONER WITH THE GOVERNMENT
OF INDIA, SIMLA.

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A PRELIMINARY REPORT ON A PARASITE FOUND IN PERSONS SUFFERING FROM ENLARGEMENT OF THE SPLEEN IN INDIA.

(Received February 14th, 1904.)

THE parasites which are the subject of this preliminary report were described in May 1903, by Major W. B. Leishman, R.A.M.C., who found them in 1900 in the examination of a film taken *post-mortem* from the spleen of a case of fever apparently contracted in Dum-Dum near Calcutta.* Major Leishman observed the constant presence of a large and a small chromatin mass in the bodies, and chiefly on this account suggested that they were possibly the remains of trypanosomata, and that similar anomalous cases of so-called malarial fever might in reality be cases of trypanosomiasis. In July 1903, Captain C. Donovan, I.M.S., reported that he had found bodies identical with those described by Leishman, in blood taken from cases by splenic puncture during life. He failed to find trypanosomes in the peripheral blood of any of his cases and the bodies appeared to be so defined and characteristic that he considered them to be a new form of parasite. He sent specimens to Major Ronald Ross, I. M. S., and to Professors Laveran and Mesnil. From his examination of the specimens Major Ross came to the conclusion that the parasite was a new one, and proposed to classify it in a new genus, Leishmania, and to call the particular parasite in question *Leishmania donovani*. In January 1904, Professor Marchand and Dr. Ledingham, influenced by the publications of Leishman, Donovan, and Ross, published an account of a case in which similar bodies had been found *post-mortem* in sections of the spleen, liver, and bone marrow, in December 1902. In the meantime Professors Laveran and Mesnil had examined the specimens sent to them by Captain Donovan and had given a systematic description of the bodies and assigned to them a zoological position. They found forms included in a substance which they took to be the body of a red blood corpuscle changed by the presence of the parasite. In addition to circular forms they found bodies which were distinctly pear-shaped, and this appeared to them to be the most typical form of the parasite. Bodies divided into two or more by a process of fission were also seen by them, and they finally came to the conclusion that the parasite was a new species of piroplasma and named it *Piroplasma donovani*. Owing to the importance of the subject I was directed to visit Madras and investigate the nature of the parasites discovered there by Captain Donovan.

* Similar parasites were discovered by Surgeon-Major D. D. Cunningham, I.M.S. in 1885. See *Scientific Memoirs by Medical Officers of the Army of India*. Part I. Printed by the Superintendent of Government Printing, India; Calcutta 1885.—Ed.

I proceeded at once to Madras and through the kindness of Major Donovan I have been able to examine many of his cases and specimens. I have also seen cases in the wards of Major Robertson and Captain Kirkpatrick, and have been able to minutely examine the tissues in three autopsies on fatal cases of the disease. I have also been able to compare the human parasites with those of two species of piroplasmata, one in calves and the other in *pariah* dogs in Madras.

I shall describe the points which I desire to bring forward in this report under the following headings :—

(1) The clinical features of the cases in which the parasites are found.

(2) The *post-mortem* appearances of cases.

(3) The importance of the disease and its relation to *kala azar*.

(4) A description of the parasites and of their relation to the red blood corpuscles and to the leucocytes.

(5) The distribution of the parasites in the body, with an account of a new method of staining sections by a modification of Romanowsky's stain.

(6) A comparison of the parasites with species of piroplasma.

(7) The nature of the so-called "zooglea mass."

1. Clinical features of cases.

Whatever may be the signs and symptoms during the early stages of the disease the final picture is extremely characteristic. The cardinal signs are—

(1) Great enlargement of the spleen.

(2) Emaciation.

(3) Irregular pyrexia.

(4) Abdominal symptoms.

To these may be added the facts that malarial parasites are very rarely found, and that the pyrexia and course of the disease are quite uninfluenced by quinine.

Enlargement of the spleen.—This is always marked and is usually much greater than in cases of malarial fever. The enormous size of the organ is, indeed, the most distinctive feature of the disease. The edge of the spleen is found far below the costal margin and most frequently reaches to the level of the umbilicus or even to the pubes. The distension of the abdomen, the emaciation, and the great splenic enlargement, give a peculiar and very characteristic appearance to the cases.

Emaciation.—In fairly advanced cases emaciation is generally present, and in cases which are proceeding to a fatal termination it is usually extreme.

Irregular pyrexia—Fever of an irregular character, reaching to 103° F. or 104° F., or more and showing no definite periodicity, is almost constantly present. In some cases it shows an undulant character, periods of high fever alternating with periods in which the rises of temperature are not so high. The temperature is not influenced by quinine.

Abdominal symptoms.—Diarrhœa and a dysenteric condition with blood and mucus in the stools are a constant feature in advanced cases. Death from peritonitis due to perforation occurred in two out of the three fatal cases which are referred to in this report. The fæces of two cases in which blood and mucus were present in the stools were examined for *amœbae* without success. The parasites were also searched for in the fæces of these cases but none were found.

Oedema of the feet is sometimes, but not constantly, present.

Pigmentation of the skin is not usually in excess of the normal, and in two cases seen by me in Eurasians with light skin there was no undue pigmentation of the skin or mucous membranes.

2. Post-mortem appearances.

These are as distinctive as the clinical signs. The following are the essential points noted at the *post-mortem* examinations of three fatal cases :—

Autopsy No. 1.—A male patient. Age about 50. Death from purulent peritonitis. The abdominal cavity contained purulent fluid; omentum adherent to the intestines; patches of congestion and lymph exudation on the small intestine.

Spleen very large, length about 9 inches; of firm consistence and not pigmented.

Liver not enlarged; considerable change, which was visible to the naked eye, had taken place in the liver substance.

Small intestine.—Patches of congested mucous membrane covered with mucus containing worm-shaped clots were scattered over the gut. A fair number of *anchylostomata* were present.

Large intestine.—The mucous membrane was greatly thickened and markedly congested. Large ulcers, one of which had perforated the gut, were present throughout the whole length of this portion oi the intestine.

The *kidneys*, *lungs*, and *heart* presented no marked changes. Some punctiform hæmorrhages were present on the parietal peritoneum and on the under surface of the arachnoid.

Autopsy No. 2.—A female patient. Age about 15. Death from broncho pneumonia following cancrum oris.

Spleen enlarged; length six inches; consistence firm; not pigmented.

Liver not enlarged but showed lobular changes.

Small intestine apparently normal. A few anchylostomes were present, also some round worms and a few whip-worms (*Trichocephalus*).

Large intestine.—Mucous membrane for the most part normal. In the sigmoid flexure and rectum numerous ulcers about the size of a split-pea were present. Many of these presented thickened and raised edges, so that they had an almost pustular appearance. In a few a central mass of altered blood pigment was present reaching as deep as the muscular coats.

The *lungs* showed patches of congestion and consolidation.

Autopsy No. 3.—A male patient. Age about 20. Death from purulent peritonitis. The abdomen contained purulent fluid.

Spleen much enlarged; length 8 inches; consistence firm; not pigmented.

Liver not enlarged but lobular changes visible. A small abscess cavity about the size of a marble was found in the left lobe; it contained soft caseous material.

Small intestine.—Patches of congested mucous membrane were present, and on the edges of the valvulæ conniventes small red dots resembling grains of Cayenne pepper (capillaries of villi) were seen. Anchylostomes were not found.

Large intestine.—The mucous membrane was greatly thickened throughout and dark pink in colour from congestion. Small erosions were present throughout the gut. Patches, several square inches in extent, showed projecting small red granulations and were covered by tenacious pink mucus. Small ulcers, often hidden by the overlapping mucous membrane, were numerous, and large ulcers extending over several square inches were situated at intervals along the gut. Perforation of the base of one of these had taken place.

The lymphatic glands in the retroperitoneal tissue were not apparently enlarged.

There were some punctiform hæmorrhages on the parietal peritoneum.

The red marrow in the sternum seemed increased in amount.

3. The importance of the disease.

Cases exhibiting the clinical picture and *post mortem* appearances described above are by no means rare, nor are they confined to Madras. Ross and others have long ago noted the presence in Bengal of cases of fever accompanied by great enlargement of the spleen in which malarial parasites could not be found. In 1901, in conjunction with Stephens and James, I examined in the hospitals of Calcutta more than 60 cases which had been diagnosed as malarial cachexia. All showed greatly enlarged spleens and an irregular, often high, temperature. The operation of spleen puncture was not performed in any of the cases, but no malarial parasites were found in the peripheral blood, and the cases in which leucocyte counts were made exhibited no increase in the large mononuclear leucocytes. The *post mortem* lesions in some of these cases were identical with those found in fatal cases of infection with the new parasites. At the time we considered that the diagnosis of malaria in such cases was made on very slender grounds. I now recognise the identity of such cases with those I have seen in Madras in which the new parasite is found. Exactly similar cases were seen by us in many parts of India, and I have no hesitation in saying that cases of infection with these parasites are clinically identical with cases diagnosed as "malarial cachexia with enlarged spleen," so well recognised throughout India.

The question naturally arises as to the relation of the cases in Madras to the disease known as *kala azar* in Assam. In the descriptions of *kala azar* stress is laid on the pigmentation of the skin, on the extreme weakness of the patients, and on the more rapidly fatal termination than in the cases of enlarged spleen seen in and about Calcutta. During my work with the Royal Society's Malaria Commission in India we did not study *kala azar*, but in the Madras cases I can detect no increase of pigmentation, and the weakness of the patients and the duration of the disease do not seem different to that in ordinary cases of so-called "malarial cachexia with enlarged spleen." I have, however, seen a specimen from the spleen of a case of *kala azar* which was sent by Dr. Bentley to Captain Donovan in which the bodies were very numerous.* If they are the cause of this disease, then the disease of Assam called *kala azar* and the common disease of India called "malarial cachexia with enlarged spleen" must be essentially of the same nature, though the one may possibly be a more severe form of the disease than the other.

* Blood films obtained from the spleens of five cases of *Kala azar* which were sent to Simla by Dr. Bentley have been examined and the new parasite has been found to be present in large numbers in all. Malarial parasites were not found in any of these films.—Ed.

4. A description of the parasites.

When the bodies are present at all, they are usually to be found in large numbers. They are seen with difficulty in fresh preparations, but can be detected and distinguished from blood platelets. They are rather more refractile than platelets and often have a faint greenish tinge. One or two more refractile masses can be made out in the body of the parasite and in many cases an appearance like a grain of pigment is seen. On careful focussing this is seen to be a minute refractile spot like "Manson's spot" in lymphocytes. The bodies are quite motionless.

In films stained by Romanowsky's method the bodies are seen with great clearness. They exhibit a remarkable uniformity in size and appearance. The majority are about 2·5 micromillimetres in diameter, but forms may be found which are a little larger, 3 to 3·5 micromillimetres, or a little smaller, 2 micromillimetres. Very occasionally still smaller bodies, 1·5 micromillimetres in diameter, may be encountered. Most of the bodies are approximately circular in outline, but very many, and especially the larger forms, are irregularly oval and very much resemble a cockle-shell in shape (Figures 7 and 8). This, indeed, appears to be the most typical shape and even in the apparently round forms an approach to this shape can be made out. Occasionally specimens are found more elongated and distinctly pear-shaped.

The bodies are very clearly outlined, and appear to possess a distinct and comparatively resistant cuticle. They retain their proper shape and are very rarely seen distorted. I have seen a body which had been crushed and from which the two chromatin masses had been squeezed out. The larger mass still lay partially within the body which had an appearance exactly resembling that of a ruptured cuticle.

The bodies are very resistant to hypotonic solutions and may be recovered by centrifugalisation from blood that has been laked by such a solution.

Except in very rare small forms which show only a single chromatin mass, the bodies invariably possess two chromatin masses—a large one staining lightly and a small one staining intensely with the red colouring matter of Romanowsky's stain.

The two chromatin masses are usually situated opposite to each other in the shorter axis of the parasite.

The small chromatin mass is usually rod-shaped, but may appear as a dot only. It is usually contained in what appears to be protoplasm displaced to the periphery of the body by the vacuole or vacuoles. It may appear close to the periphery or centrally situated. In the latter case it is probably in reality

on the periphery also, since it seems to be generally situated in some part of an arc lying immediately beneath the cuticle and in a plane at right angles to the length of the body when this can be made out. There is often a " tail " joining it to the larger mass. As a rule, this appears to be formed only of a strand of protoplasm. On one or two occasions a minute chromatin particle appeared to be situated in it.

The large chromatin mass stains much less darkly than the small one and is obviously of a quite different nature. It frequently stains so as to show a darker and lighter portion. It is very commonly bilobed, and often heart-shaped, or formed of two oval masses lying in contact. In some cases bodies are seen in which the large chromatin mass stains very faintly although other bodies in the same field stain normally. The large chromatin mass is situated near what would be the hinge in the cockle shell shaped forms, and in the thicker end of the pear-shaped forms.

The body substance usually stains pink by the method employed (fixation in alcohol, and staining by watery solutions of eosin and Romanowsky blue). In some specimens the protoplasm stains faintly blue. In a few forms a uniform pink staining hyaline substance is seen filling the body of the parasite. In the majority of bodies one or two vacuoles are present which in many cases have pushed the pink staining substance to the periphery. A ridge of protoplasm left between the vacuoles forms the " tail," sometimes seen joining the two chromatin masses. The protoplasm is not as a rule uniformly disposed around the periphery, but is collected in greater amount on one or other side of the small chromatin mass.

The bodies have a very regular external form and arrangement of parts. A cockle shell will best represent the shape of the parasite. In the thicker portion, where the body of the cockle is placed, lies the large chromatin mass, often divided into two lobes or even into two separate bodies—one in each valve. Near the centre of the convex free edge of the valves and lying partly in each is the small chromatin rod. The forms seen lying flat appear as in figs. 7 and 8. The pear shaped forms are these seen edgeways. In many aspects the bodies appear nearly circular. Various views of our imaginary body will represent almost all the arrangements of the chromatin masses and the shapes assumed by the parasite in films (see descriptive diagram).

Developmental forms.—Forms may be seen which appear to show division of the bodies into two. Appearances showing both longitudinal and transverse fission are seen. In the former, two pear shaped bodies are found lying side by side. The large chromatin masses are situated in the thick end of the pear and the small rod bodies at the thinner ends. Division occurs first at the thick

end, and the small chromatin mass may often be seen undivided when the larger masses are widely separated.

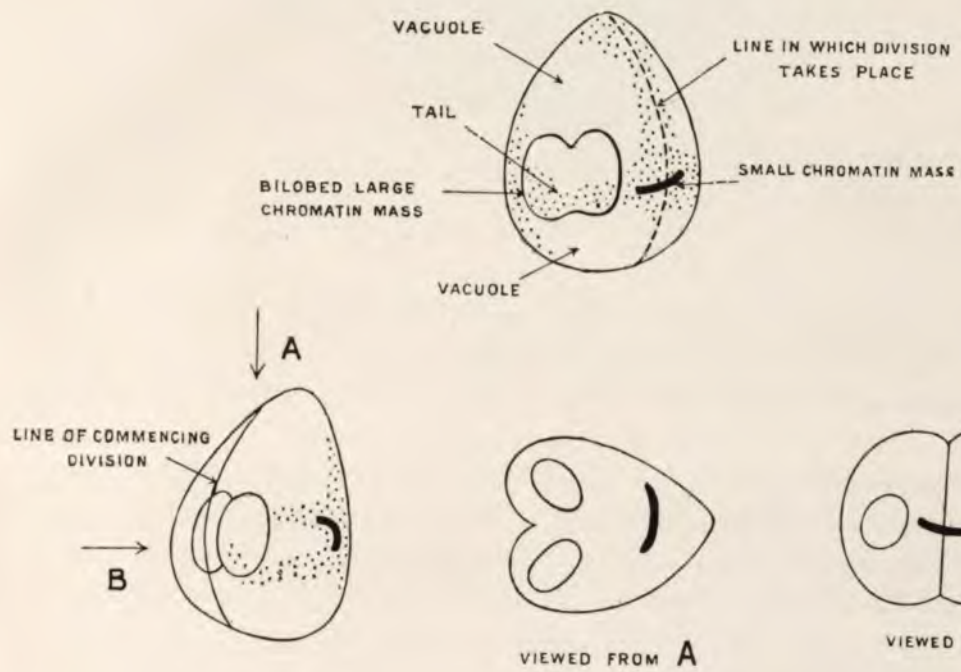
In the forms which apparently show transverse division the two large chromatin masses are at opposite poles separated by the two (or the as yet undivided) small masses. Both these appearances represent the same process from different points of view (see accompanying diagram). Division takes place in the line of separation of what would be the two valves of the shell form. Seen from one direction (A) the double pear-shaped form is seen. Seen from B. apparent transverse division. The bilobed, heart-shaped and double forms of the chromatin mass appear to be early stages of the same process.

In addition to the above, forms are seen showing division into three or more bodies. These forms are more or less circular, of larger size than the largest of the single forms, and may be as large as a red blood corpuscle. In typical specimens the large chromatin masses are arranged peripherally, whilst the small rod shaped masses are nearer the centre. The formation of from 3 to 6 bodies is most common.

Asexual and sexual forms.—No appearances have been seen by me which seem to point to the presence of two forms of the parasite. Variations in the bodies appear to be insignificant and to depend mostly upon the point of view from which they are seen. The two most distinct forms are those with and those without vacuoles. The forms showing uniform pink body substance are often quite large. Another form is that with a very faintly staining chromatin mass.

Relation of the parasites to the red cell.—In ordinary preparations taken by splenic puncture or in smears taken *post mortem*, many of the bodies are free and found scattered over the slide. Other forms are seen included in leucocytes and macrophages. Others again are found surrounded by some substance which stains faintly blue and appears granular or reticular in structure. Bodies lying in such material may occur singly or in numbers up to 10 or more. According to Laveran this blue staining substance is the red cell rapidly disorganised by the presence of the parasite. Ross mentions it only as a matrix.

Most of the apparently free bodies seen in films are, I believe, derived from the leucocytes, and especially from enormous cells which are crowded with the bodies and which readily become broken up. In many cases the bodies may be seen to have been carried in streaks from such cells by the action of the slide or needle used in spreading the film. Free bodies are generally scanty when blood flows readily into the syringe during puncture. When this is the case very few splenic cells are seen in the preparations and at the same time very few parasites. They are more abundant when actual splenic pulp is used than when the films are made from blood taken from the spleen. In sections enormous numbers of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bodies are seen included in cells, but I have searched in vain for free bodies, and if present at all they must be in very small number only.

The blue staining substance in which some of the bodies are seen is of great interest, and upon the interpretation of its nature the zoological position assigned to the parasite depends. The appearance of this substance varies slightly in different preparations. It stains faintly or more darkly blue and shows a hyaline, finely granular or reticular structure. It may be, and often is, about the size of a red cell but it may be very much smaller and it may be larger. If small, it usually contains only a single body. When five or more bodies are present the size exceeds that of a red cell. The outline is often very distinct, but it is sometimes blurred or quite irregular. In some cases the resemblance to a red cell altered by the simple tertian parasite is very marked. Distinct stippling is not, however, seen. Bodies resembling these, but not containing parasites, are generally numerous in preparations showing included forms. If blood containing the bodies is placed in a hypotonic solution of ammonium oxalate and the centrifugalised sediment examined, the blue staining material around the bodies becomes more clearly defined and stains more darkly blue. At the same time the protoplasm of the mononuclear leucocytes shews a marked tendency to budding and separation from the cell and stains more clearly. But the red blood corpuscles are seen only as ghosts.

In one of the cases examined by us during life and *post mortem*, bodies resembling altered blood cells contained undoubted malarial pigment. In the large macrophages similar pigment was seen included in the same cell as the parasites.

I think there can be no doubt, therefore, that very many of the blue staining bodies in which included parasites are seen, are pieces of detached cell protoplasm derived from the large mononuclear cells and especially from the macrophages. In many preparations the process of detachment may be observed, and buds of protoplasm containing parasites are seen projecting from the cell mass. The process appears to be assisted by the addition of hypotonic solutions and may be largely due to the fluid always present in the syringe when used for spleen puncture.

Whether the blue substance is always of leucocytic nature, whether some of the bodies seen are really red cells (Fig. 19), I hesitate to say; but however striking the resemblance to the red cell may have been, we have always found the protoplasm of the leucocytes to be similarly stained.

Relation of the parasites to leucocytes.—A most striking feature in connection with the bodies is their presence in great numbers in an unchanged state in the

leucocytes. They are found especially in the large mononuclear cells. Such cells contain from one to twenty bodies. The polymorphonuclear cells also often contain parasites, but rarely more than two or three. I have seen single bodies in eosinophil cells and in finely eosinophil myelocytes ; also in the nucleus of some cells, but I am not sure whether these latter were not superimposed bodies. In addition to the leucocytes noted above, large cells with spherical nuclei, quite distinct from the large mononuclear forms, are seen in preparations. They are usually packed with parasites and have a most characteristic appearance. They usually contain from 20 to 100 bodies, but may contain as many as 250. Some of these cells are of gigantic size, but become broken up in films and are seen to advantage only in sections.

Bodies included in the leucocytes and in the large cells described above show no signs of intracellular digestion. They stain very clearly, and when liberated from the cell show a clear outline. They do not appear to lie in vacuoles in the cell protoplasm.

5. The distribution of the parasite in the body.

a. Peripheral blood.—I have been unable to find the bodies in the peripheral blood even of cases in which they occurred abundantly in the spleen. The examination of peripheral blood taken at 6 P. M. and at midnight as well as of blood taken during high fever (104° F.) has been negative. I have not so far been able to detect the parasites in the peripheral blood even after centrifugalisation.

b. Blood from the deeper vessels.—The parasites were not found in blood taken *post mortem* from the deeper vessels of the abdominal wall.

c. Blood from the spleen.—When blood from the spleen flows freely into the syringe the parasites are found in it, but only in small numbers.

d. Blood from the liver and portal vein.—In blood from the liver the parasites may be abundant. In blood taken *post mortem* from the portal vein a considerable number of the bodies were found, especially in the large mononuclear cells.

e. Petechiae from the arachnoid.—In petechiæ from the arachnoid a fair number of the bodies were seen, all included in polymorphonuclear cells.

f. In the tissues.—In studying the organism in the tissues I have made use of a modification of Romanowsky's method of staining. It is founded upon the observation of Stephens that the characteristic staining with Romanowsky's stain could be as well obtained by

placing the films first in the eosin solution and then in the methylene blue solution as by mixing the two solutions together before pouring them on the slide.

So far as I am aware, it has not yet been found possible to stain organisms in sections by Romanowsky's method. Treated in the ordinary way, sections stain merely with the methylene blue, and the nuclei show no trace of the characteristic red body of the Romanowsky stain. Films treated first by alcohol, xylol, and the paraffin bath, still stain quite readily, so that the reason why sections do not stain appears to be a physical one. Stephens found that Romanowsky staining was developed when films were placed first in eosin and then in the methylene blue solution, and we were accustomed to use this method when dealing with large numbers of films. By employing this method of saturating sections first with eosin and then placing them in the methylene blue solution I have been able to stain the nuclei and included parasites with the characteristic red of Romanowsky's stain. The following is an account of the method.

Fix the tissues in absolute alcohol or saturated aqueous solution of perchloride of mercury. Use small slabs 1 to 2 millimetres thick placed upon paper so that they may harden flat. Remove carefully all adherent paper fibre and embed in paraffin, using, after complete dehydration (1 to 4 hours in absolute alcohol) only 15 minutes in xylol and at most 20 minutes in hard paraffin.

Cut thin sections. Flatten by floating out on water not quite hot enough to melt paraffin. Float on to a slide. Remove the excess of water and press firmly with filter paper. No fixative is necessary if this be done. Dry a few seconds at a height above the flame just sufficient not to melt the paraffin. Melt the paraffin and treat as usual with xylol, absolute alcohol, and water.

Stain for 10 to 15 minutes in 1—1,000 eosin (Höchst, B. A. Grübler).

Pour off the excess of eosin and press the section with filter paper. Add an excess of the methylene blue solution prepared according to the following formula and allow to stain for 15-20 minutes.

100 c. c. of methylene blue (pure medicinal, Grübler) solution in distilled water.

5 c. c. of a 10 per cent. solution of chemically pure sodium carbonate.

Leave in tropical sun until a strong red colour is seen on shaking.

Dilute 25 times for use.

Pour off the excess of stain and wash rapidly in 70 per cent. alcohol. The washing in alcohol should be quite momentary. If the washing is too prolonged too much red colour will be removed, and if too short some precipitate will be left. Transfer as rapidly as possible to water.

If, after washing, the section is dark purple or blue, wash in 1 to 400 acetic acid in distilled water, and at once plunge into water. The acetic acid removes the excess of methylene blue staining and makes the tissue much clearer. If too long washing is given, the red colour will also be removed. If the section is light reddish or light purple after washing with alcohol the acetic acid bath is unnecessary.

Allow the section to dry upon the slide. It is on account of the necessity for this procedure that thin sections must be used. In the case of very thin sections shrinkage is negligable, but in thick sections it is considerable.

Mount in Canada balsam or keep as a film and examine merely by placing a drop of cedar oil on the section.

Quite unshrunken tissues may be examined after staining by washing and mounting in glycerine. The result is useless as a permanent preparation since the glycerine dissolves out the red stain, but it is useful for temporarily displaying the relation of parasites to the tissues.

Several kinds of tissues may be examined in this way, and the parasites displayed by spreading portions of the tissues upon a slide, drying, fixing in alcohol, and staining as above. Omentum, teased muscle, connective and other tissues give good results. Micro-organisms are very well shown when present.

The changes in the organs and the distribution of the parasite in the tissues were similar in all three fatal cases.

Films made from the *liver substance* showed many bodies free and in leucocytes. The most striking feature was the presence of large leucocytic cells crowded with the bodies. The liver cells were free from parasites.

Sections of the liver showed very clearly the relation of the parasites to the tissues. The lobular capillaries were dilated and the liver cells, especially in certain areas, were much atrophied. In the capillaries were seen numerous large cells crowded with parasites. The bodies of these cells were usually situated in the lumen of the capillary, but attached by several processes to the capillary wall. In many cases they were applied closely and flattened against the capillary wall. The protoplasm of these cells was often retracted so as to form a globular mass, but more usually was stretched out along the capillary in a very characteristic way. The appearance of these cells irresistibly suggests large amœbæ crawling about the capillaries. In some parts of the sections these cells were extremely numerous, forming the bulk of the tissue. In one of the cases the cells were so crowded with bodies that their nuclei were often hidden. The exact nature of these large cells is doubtful, but they appear to be identical with the large

macrophages seen in the organs in cases of malaria. In one of the cases malarial pigment actually occurred in some of these cells along with the bodies under discussion. I have never seen such large cells in fatal cases of malaria, and their great development appears to be a feature of infection with the new parasites.

In sections the bodies lying in the cell protoplasm showed very distinctly the two chromatin masses and a clear space. This space appeared at first to be a vacuole in which the parasite lay, but it is undoubtedly the body of the parasite itself. The chromatin masses frequently occupied exactly opposite poles. The outline of the intervening space was always in this case remarkably circular. The appearance always strongly suggested a body resistant to shrinkage and change of shape.

The bodies were frequently seen singly or in small clusters in a pink stroma. This stroma could nearly always be made out to be a severed process of a macrophage.

No bodies were found in red blood corpuscles (stained bright red by the method employed) or lying free.

Bodies were occasionally seen lying in leucocytes in the lumen of branches of the portal vein. They were never numerous in this situation.

The epithelium of the small *bile ducts* was unchanged. The bodies were not present in the bile.

In films and sections of the *spleen* similar cells to those in the liver capillaries were very conspicuous and contained the great majority of the parasites. Large mononuclear cells containing parasites were more abundant than in the liver. Occasionally a single parasite was seen in close connection with the endothelium or stroma cells. In the red cells no forms could be detected. Free forms were never seen.

In films of the *red bone marrow* the bodies were not so numerous in any of the cases as in the liver and spleen. In smears made with care to avoid as far as possible rupturing the large cells, the bodies were almost confined to the macrophages, some of which contained an immense number of them. They were also contained in large mononuclear cells and isolated specimens were present in the polymorphonuclear cells and finely grained myelocytes. No forms could be seen in undoubted red cells. Megaloblasts were present.

The constant presence of intestinal lesions and the marked character of the changes found make the examination of *the intestine* of great importance. Smears were taken of mucous membrane at the edge of ulcers and from congested patches, as well as from the small red granulations noted in the description of autopsy No 3. In this autopsy the tissues were preserved within one hour of death so that they had changed but little.

The examination of sections shows that, prior to ulceration, there is a formation of granulation tissue in the mucosa, which may eventually be entirely replaced by new tissue. The latter then encroaches upon the crypts and surface epithelium, destroys them, and either projects as fungating granulations of

various sizes, or undergoes necrosis, thus leading to the formation of ulcers reaching to the muscular coats.

In a number of smears made from projecting granulations and from the mucous membrane in the early stages of infiltration I have found the parasites in large numbers. They occur (as in the spleen, liver, and bone marrow) in large cells with round or oval nuclei. In some of the films these cells were almost as numerous as in the spleen, and many of them were so crowded with the parasites that their nuclei could be seen only with difficulty. In many instances they appeared to be lying in close connection with the crypts, and in some sections they were situated in the tissue in the immediate neighbourhood of crypts undergoing destruction.

The intestinal ulceration, which is so marked a feature of infection with the new parasite, appears, therefore, to be closely analogous with the condition found by Wright in cases of tropical ulcer. Moreover, the formation of new tissue characterised by the presence, in such diverse situations as the skin and large intestine, of large cells containing the parasites, has a close resemblance to the morbid processes found in the granulomata

In all the cases a very large curved micro-organism was present in great numbers, and in one case this body was seen in nearly pure culture in the crypts. These micro-organisms have a single chromatin mass placed near the centre of the body. One end is generally more pointed than the other and gives to the body somewhat the appearance of a sporozoit. I have seen these bodies dividing by transverse fission; they appear to be bacteria. In the unstriped muscle of the submucosa, especially near the bases of ulcers, were seen many collections of bodies 1-2 micromillimetres in diameter staining an intense blue and lying in elongated spaces to one side of the nucleus of the muscle fibre. They appeared polygonal in shape and did not look like other cocci seen in the tissue. We were unable to demonstrate any chromatin in them and are unaware of their nature.

The endothelium of the vessels in one case was much thickened and showed a curious hyaline appearance.

In the *small intestine* no parasites were found nor any condition worthy of note. Smears from the *lymphatic glands* in the retroperitoneal tissue were examined, but no parasites were found.

No parasites were found in the *kidneys* in the one case in which these were examined. The *supra-renal bodies*, the *brain* substance, the *bile*, *peritoneal fluid* and the *lungs* appeared free from the bodies.

Particular attention was given to the *muscles*. Muscle fibres from the larynx, œsophagus, psoas, levator ani and pectorals and intercostal muscles were spread upon slides and stained by the modified Romanowsky method. The nuclei of the muscle fibres were beautifully displayed, but no parasites or other unusual conditions were seen.

Large masses of muscle as well as the œsophagus and intestines were examined with a lens but no bodies resembling sarcocysts were seen.

6. A comparison of the bodies with certain species of Piroplasma.

I have been able to compare the bodies in question with a species of piroplasma common in calves in Madras.

Major Donovan first pointed out to me the presence of this parasite in calves of the district. I have since found 6 out of 24 apparently healthy calves infected. Of these all but one showed small bacillary forms and forms somewhat resembling the malarial parasite. In one calf large typical pear shaped forms were also present. I have also found in the *pariah* dogs of Madras a species of piroplasma in which large forms are present.

In both species the large forms show what appear to be essential differences to the bodies occurring in the human infection.

(a). Forms are readily demonstrated in the red cells. They occur singly or in twos or in fours and the bigeminal division is most distinct.

(b). They have a granular protoplasm staining blue and their outline is not suggestive of a body with a capsule.

(*c*) There is no appearance at all like the highly distinctive double chromatin masses. In cases of infection with the malignant tertian malarial parasite small forms are often seen containing two chromatin masses (fig. 28), but the appearance and arrangement of these is totally unlike that of the new parasite. In piroplasma of the calf and of the dog the chromatin resembles much more that of a malarial parasite than it does that of the new bodies.

(*d*) In piroplasma various stages of the parasite have always been seen by me in the same animal. In the large forms of piroplasma, fission takes place in a way unlike that seen in the new human parasite. In piroplasma the forms stretch across the cell to form first a long ellipse and then separate by the formation of a constriction. In piroplasma it is easy to see development in progress. In the human parasite the small number of division forms compared to the number of bodies present is remarkable.

(*e*) The complete absence of the new human parasite in the peripheral blood in apparently all cases, is unlike piroplasma, which is found readily enough in some form or another in the peripheral blood.

As a result of my researches I have shown that the distribution of the parasite in the body is very limited. Even in the liver and spleen it is chiefly as a result of an intense phagocytosis that we see the bodies. The resistance of the bodies to intracellular digestion appears to lead to the storing up of the parasite in the large macrophages of the liver and spleen. Apart from the above two organs and the bone marrow I have found the bodies only in the mucous membrane and sub-mucosa of the large intestine (especially in the tissues

in the immediate neighbourhood of ulcers), in the blood from the portal vein and in punctiform hæmorrhages.

The origin of the bodies seems to me very obscure, and I am not satisfied that they are a form inhabiting the red cell at any stage.

I can trace only a superficial resemblance to piroplasma in cattle and in the dog.

Since Laveran has given so decided a verdict I can only say that the differences between the two forms appear so great that strong evidence of their affinity must be forthcoming before the new parasite can be placed in the genus *Piroplasma.*

If the parasite were present in the red blood cells, if it presented a distinctly pear shaped form, and if there were evidence of binary fission, the grounds for doing so would no doubt be sufficient. But most of the bodies resembling red cells are undoubtedly the fragmented protoplasm of leucocytes and macrophages, and the fact that the bodies are present in red cells cannot be said to have been proved. The pear-shaped form seems to be but one aspect of bodies which have a shape something like a cockle-shell. The binary fission of the bodies seems to me to be quite different from that seen in piroplasmata.

Until the relation or want of relation between the bodies and the red blood cell is clearly proven I think it premature to make any remarks as to their zoological affinities. The unyielding shape, the apparent possession of a cuticle, and the arrangement of the two chromatin masses, seem to me to point to an organism very distinct from one of the genus *Piroplasma*, and to be very suggestive of the spores of a Microsporidian.

7. The nature of the so-called "Zooglea Mass."

Manson and Low have described the substance in which the parasites are frequently contained as a "Zooglea mass." That the substance is the protoplasm of the large cells in the liver and spleen capillaries is evident from the examination of sections. That these large cells are not themselves parasitic is I think demonstrated by their resemblance to the macrophages in malaria and by the presence in specimens from one case of malarial pigment in these cells along with the bodies in question (Fig. 27).

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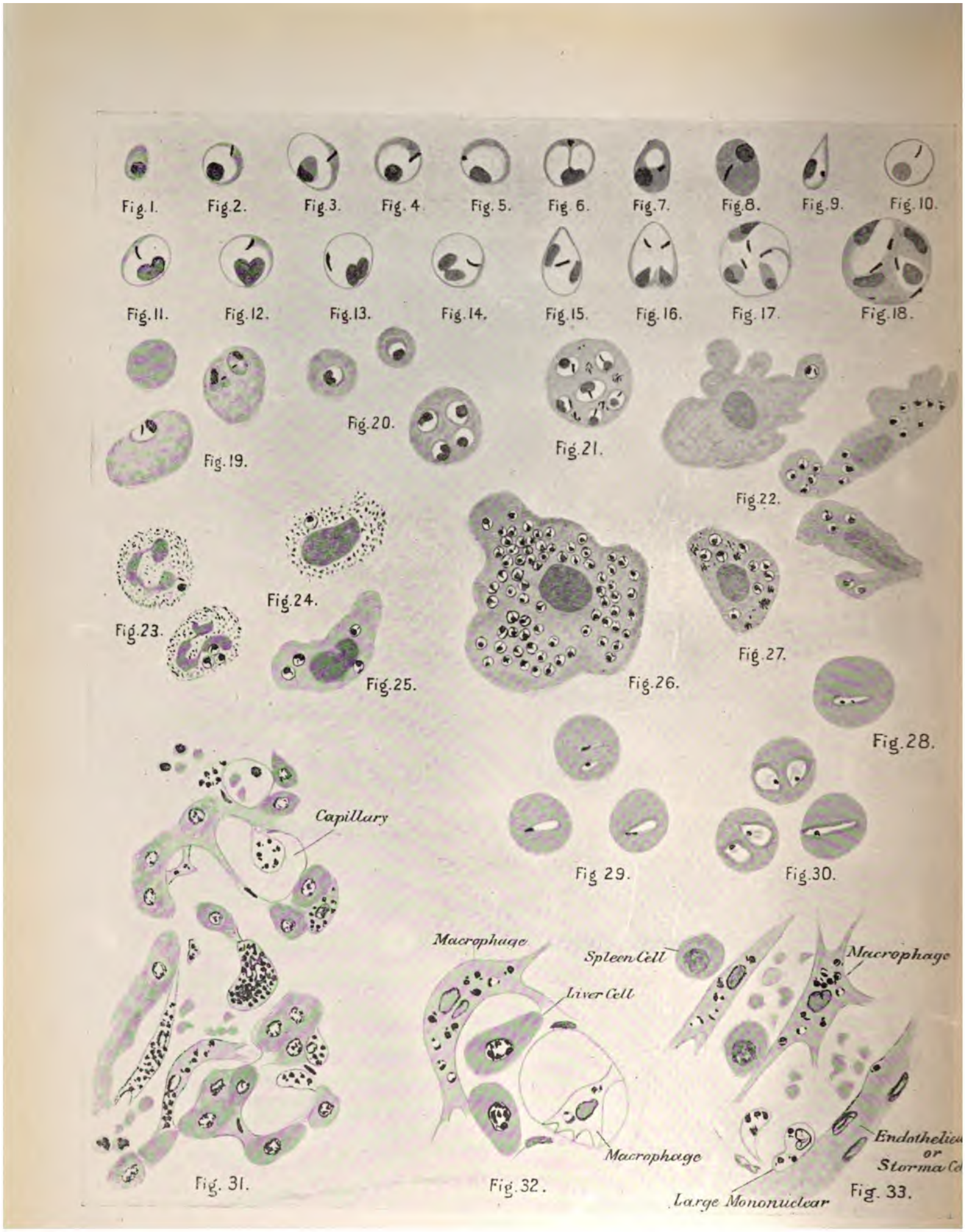
Fig. 1.
Fig. 2.
Fig. 3.
Fig. 4.
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Fig. 6.
Fig. 7.
Fig. 8.
Fig. 9.
Fig. 10.
Fig. 11.
Fig. 12.
Fig. 13.
Fig. 14.
Fig. 15.
Fig. 16.
Fig. 17.
Fig. 18.
Fig. 19.
Fig. 20.
Fig. 21.
Fig. 22.
Fig. 23.
Fig. 24.
Fig. 25.
Fig. 26.
Fig. 27.
Fig. 28.
Capillary
Fig 29.
Fig. 30.
Macrophage
Spleen Cell
Liver Cell
Macrophage
Fig. 31.
Fig. 32.
Macrophage
Endothelium
or
Stroma Cell
Large Mononuclear
Fig. 33.

Explanation of Plate.

Fig. 1.—Small form of parasite without vacuole or secondary chromatin body. Rarely seen.

Fig. 2.—Small form of parasite.

Fig. 3.—Large form of parasite.

Fig. 4.—Parasite showing rod shaped small chromatin mass.

Fig. 5.—Parasite showing small chromatin mass as a dot only.

Fig. 6.—Parasite showing "tail" joining chromatin masses.

Fig. 7.—Cockle shell shape with vacuole.

Fig. 8.— Ditto with pink staining body substance and no vacuole.

Fig. 9.—Pear shaped form.

Fig. 10.—Form with faintly staining large chromatin mass.

Fig. 11.—Form showing bilobed chromatin mass.

Fig. 12.— Ditto heart-shaped chromatin mass.

Fig. 13.—Similar form to the last; seen in another direction.

Fig. 14.—Form with double large chromatin mass.

Fig. 15.—Longitudinal division into two forms.

Fig. 16.—Transverse division into two forms.

Fig. 17.—Developmental form showing the formation of 3 bodies.

Fig. 18.—Developmental form showing the formation of many bodies.

Fig. 19.—Forms apparently in altered red blood corpuscles.

Fig. 20.—Forms in bodies after treatment of blood with hypotonic ammonium oxalate solution.

Fig. 21.—Pigment in apparent red cell.

Fig. 22.—Showing formation of apparent red cell bodies from macrophages and leucocytes.

Fig. 23.—Parasites in polymorphonuclear leucocytes.

Fig. 24.—Parasite in a myelocyte.

Fig. 25.—Parasites in a large mononuclear leucocyte.

Fig. 26.—A macrophage containing the parasites.

Fig. 27.—A macrophage containing malarial pigment in addition to the parasites.

Fig. 28.—Malignant tertian malaria. Young form showing two chromatin dots.

Fig. 29.—Small forms of bovine piroplasma.

Fig. 30.—Large forms of bovine and canine piroplasma.

Fig. 31.—Section of liver stained by the modified Romanowsky method showing macrophages in the liver capillaries.

Fig. 32.—Macrophages in the liver capillaries containing parasitic bodies.

Fig. 33.—Section of spleen showing the parasites in macrophages and in large mononuclear cells; a parasite is seen in connection with an endothelium or stroma cell.

h.

No. 8.

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